# Comparison of ARCHITECT chemiluminiscent microparticle immunoassay for determination of Troponin I in serum with AXYM MEIA technology

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## Abstract

**Introduction**: The aim of this study was determination of troponin I at serum using Architect (Abbott) and AxSYM System (Abbott). Troponin is regulatory subunit of the troponin complex associate with actin filament within muscle cells and it is a marker for diagnosis of myocardial damage.

**Methods**: We used Architect STAT chemiluminescent microparticle immunoassay (CMIA) and AxSYM microparticle Enzyme Immunoassay (MEIA), techniques for quantitative determination of cardiac TnI in human serum or plasma. At our study we have proved precision, reproducibility and accuracy from both methods. The investigation included patients (n=119) who have myocardial infarction or ischemic heart damage and were treated at cardiology, emergency, internal medicine and neurology unit in Clinical Center University in Sarajevo.

**Results**: The precision for three controls using Architect STAT TnI asssay technology were 3.6 – 5.2 % and reproducibility was 3.7 to 5.6 %. The AxSYM STAT TnI has precision for three controls 4.3–6.6 % and reproducibility was from 4.8 to 7.8 %. We have got very good correlation between Architect and AxSYM technology r = 0.999 in the investigation of troponin I in serum.

**Conclusions**: We can conclude that chemiluminescent troponin assay I (Architect) showed good analytical performance and gave new possibility at troponin I determination. 

*Keywords: Troponin, MEIA and CMIA*

## Introduction

Acute myocardial infarction is a major cause of death and disability. Approximately 15 million patients per year in the United States and Europe present to the emergency department with chest pain or other symptoms suggestive of acute myocardial infarction (1). Clinical studies have demonstrated the release of troponin concentration (cTnI) into the blood steam within hours following myocardial infarction (MI) or ischemic damage. Elevated levels of cTnI (above the values established for non-MI specimens) are detectable in serum within 4 to 6 hours after the onset of chest pain, reach peak concentration in approximately after 8 to 28 hours, and remain elevated for 3 to 10 days following MI. Cardiac troponin is the preferred biomarker for the detection of myocardial injury based on improved sensitivity and superior tissue-specificity compared to other available biomarkers of necrosis, including CK-MB, myoglobin, lactate dehydrogenase, and others. The high specificity of cTnI measurements is beneficial in identify cardiac injury for clinical conditions involving skeletal muscle injury resulting from surgery, trauma or muscular disease (2). The Joint European Society of Cardiology/American College of Cardiology/American Heart Association/ World Heart Federation Task Force redefinition of acute myocardial infarction (AMI) is predicated on the detection of increase or decrease of cardiac troponin (cTn), with at least 1 concentration above the 99th presence reference

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value in patients with evidence of myocardial ischemia. Blood samples for measurement of cTn are recommended to be drawn at presentation and 6-9 h later to optimize clinical sensitivity for ruling in AMI (3,4). The Architect STAT chemiluminescent microparticle immunoassay (CMIA) and AxSYM microparticle Enzyme Immunoassay (MEIA), are techniques for quantitative determination of cardiac TnI in human serum or plasma. Immunoassays utilize one or more select antibodies to detect analytes of interest. Analytes being measured may be those that are naturally presented in the body, those that the body produced but are not typically present (such as troponin antigen). At the CMIA a chemiluminescent label conjugated to the antibody or antigen, and it produces light when combined with its substrate. This method is very similar to MEIA, though the chemiluminescent reaction offers high sensitivity and ease measurement. A noncompetive sandwich format yields results that are directly proportional to the amount of analyte present (5,6). Using patient samples collected in our laboratory we analyzed troponin concentration by CMIA and MEIA technology methods and compared the results.

## Methods

*Patients*

The patient samples of blood were collected in serum separation Vacutainer test tubes (Beckton Dickinson, Rutherford, NJ 07,070 U.S.) in volume of 3.5 mL. We used test tubes with gel. Serum samples were obtained by centrifugation at 3000 rpm using centrifuge (Sigma 4-10). After centrifuging, serum concentration of TnI was determined. The investigation was done respecting ethical standards in the Helsinki Declaration. The investigation included patients (n=119) in period from February till May in 2008. The patients who have myocardial infarction or ischemic heart damage were treated at cardiology, emergency, internal medicine and neurology unit in Clinical Centre University in Sarajevo.

*Chemiluminescent microparticle immunoassay – CMIA*

All immunoassays require the use of labeled material in order to measure the amount of antigen or antibody. A label is a molecule that will react as a part of the assay, so a change in signal can be measured in the blood after added reagent solution. CMIA is noncompetitive sandwich assay technology to measure analytes. The amount of signal is directly proportional to the amount of analyte present in the sample. Architect STAT Troponin I assay is two-step immunoassay to determine the presence TnI in human serum using CMIA technology. In the first step, sample, assay diluent and anti-troponin-I-antibody-coated paramagnetic particles are combined. TnI present in the sample binds to the anti-troponin-I coated microparticles. After incubation and wash, anti-troponin-I-acridinium-labeled conjugate is added in the second step. Following another incubation and wash, pre-trigger and trigger solutions are then added to the reaction mixture. The pre-trigger solution (hydrogen peroxide) performs the following functions: 1) Creates an acidic environment to prevent early release of energy (light emission), 2) Helps to keep microparticles from clumping, 3) Splits acridinium dye off the conjugate bound to the microparticle complex. This action prepares the acridinium dye for the next step. The trigger solution (sodium hydroxide) dispenses to the reaction mixture. The acridinium undergoes an oxidative reaction when exposed to peroxide and an alkaline solution. This reaction causes the chemiluminescent reaction to occur. N-methylacridone forms and releases energy (light emission) as it returns to its ground state. The resulting chemiluminescent reaction is measured as relative light units (RLU). A direct relationship exists between the amount of TnI in the sample and RLU detected by Architect System optics (2,5).

*Microparticle enzyme immunoassay - MEIA*

MEIA is an immunoassay method that utilizes the isolation of antibody/antigen complexes on solid phase surface of small beads called microparticles. It is automate technology for measurement of large molecules such as markers associate with cardiac testing. The proces of the MEIA technology includes: Microparticles coated with anti-analyte antibodies and sample are incubated together to form reaction mixture. 1) An aliquot of the reaction mixture is transfer to the glass fiber matrix. 2) Alkaline phosphatase-labeled anti-analyte antibodies are allowed

to bind to the microparticle complex. 3) The substrate 4-methylumbelliferyl phosphate (MUP) is added to the matrix. The fluorescent product, methylumbelliferone (MU) is measured. The flourescent product is measured by MEIA optical assemby (4,5). The AxSym dynamic range is 0.02-22.8 μg/L and imprecision (10% CV): 0.16-0.56 μg/L. The Architect cTnI dynamic range is 0.01-50 μg/L and imprecision (10% CV): 0.032-0.055 μg/L (7). The patients specimens for AxSym greater than 22.78 ng/mL or Architect greater than 50 ng/mL we used dilution protocol. The CMIA is new immunochemistry technique with analytical sensitivity ≤ 0.01 for cTnI detection in serum compared with MEIA with analytical sensitivity ≤ 0.02. The advantages of CIMA is detection of lowest concentration of troponin that can be measured at patents serum after MI (2,3). The reference range for TnI in serum is 0.00-0.40 ng/mL.

*Quality control*

The low, medium and high TnI controls of commercially available Architect ABBOTT and AxSYM ABBOTT were used. The precision (intra-day variation) was tested by measuring (n=20) of three different controls of TnI. The reproducibility (inter-day variation) for same controls was tested all controls once a day over 10 consecutive days. The accuracy of measuring was tested in 119 of serum patient who were determined TnI. Measures were obtained by Architect CMIA and AxSYM MEIA technology.

*Statistical analysis*

The results were statistically analyzed using NCSS and statistical software SPSS version 12.0 software. Determined by the average value ($\bar{\chi}$), standard deviation (SD), Pearson correlation coefficient (r), equations of linear regression and Student t test with statistical significance level of $p < 0.05$.

## Results

*Quality control testing*

Three controls low, medium and high Abbott technology (n = 20) were measured for quality control testing. Measurements were done during 10 days period. The average value ($\bar{\chi}$), standard deviation (SD) and coefficient of variation (CV) are shown in Table 1. The precision has coefficient of variation (CV) for three controls using Architect STAT TnI assay technology were 3.6 – 5.2 %. Reproducibility was determined by running controls in the morning over 10 consecutive day. Coefficient of variation (CV) for the reproducibility of TnI assay varied from 3.7 to 5.6 %. The precision has coefficient of variation (CV) for three controls using AxSYM STAT TnI assay technology were 4.3–6.6 %. Reproducibility was determined by running controls in the morning over 10 consecutive day. Coefficient of variation (CV) for the reproducibility of TnI assay varied from 4.8 to 7.8 %.

**TABLE 1.** Quality control testing

| Concentration spiked (ng/mL) | Concentration found intra-day (mean SD, n= 20) (ng/mL) | Precision intra-day (%) | Concentration found inter-day (mean SD, n= 20) (ng/mL) | Reproducibility (%) |
|---|---|---|---|---|
| Architect Troponin I assay CMIA technology | | | | |
| 0.145 | 0.155 ± 0.01 | 5.3 | 0.144 ± 0.03 | 5.6 |
| 0.580 | 0.632 ± 0.03 | 3.9 | 0.577 ± 0.09 | 4.9 |
| 15.67 | 16.03 ± 0.82 | 3.6 | 15.93 ± 2.49 | 3.7 |
| AxSYM Troponin I assay MEIA technology | | | | |
| 0.28 | 0.30± 0.017 | 4.3 | 0.26± 0.026 | 4.8 |
| 1.14 | 1.15 ± 0.054 | 4.7 | 1.11± 0.047 | 5.2 |
| 9.49 | 9.44 ± 0.627 | 6.6 | 9.38± 0.728 | 7.8 |

*Accurancy testing*

We compared TnI concentration measured in 119 blood serum by Architect CMIA and AxSYM MEIA technology. The results of the comparison between Architect CMIA and AxSYM MEIA technology analysis are shown in Figure 1. Sizable correlation was noted between Architect and AxSYM technology in the investigation of 119 blood samplers (r = 0.999). Regression equation revealed a slope of 0.9187 and a y axis intercept of 0.077. The difference between the methods was statistically significant for $p < 0.05$ according Student t-test. The Architect STAT TnI assay had a limit of detection of 0.004 μg/L and a CV of 10% at concentrations approaching 0.03 μg/L. The concentration of serum TnI using Architect CMIA is higher than AxSYM MEIA technology. Ten of the 119 samples (in 5 patients) were likely to be true AxSYM negatives because there were no

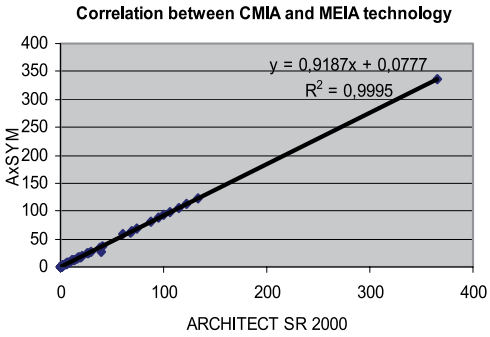


**FIGURE 1.** Comparison of TnI concentration (ng/mL) in serum measured by Architect CMIA (x-axis)and AxSYM MEIA technology (y-axis).

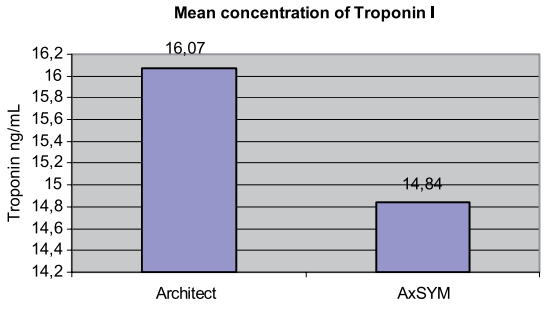


**FIGURE 2.** The comparison of Troponin I in patient serum using different methods.

detected cardiac events during follow-up. The Architect values in these 10 samples ranged from 0.04 to 0.09 μg/L. These findings highlight the potential of the Architect assay to reclassify patients previously labeled as "normal". The mean concentration of TnI by patients with no detected cardiac events in Architect assay was 0.005 μg/L and in AXSYM assay was 0.000 g/L. The average concentration of TnI in serum from all patients in study measured by Architect assay was 16.07 μg/L and in AXSYM assay was 14.84 μg/L. The results of the mean concentration of Architect CMIA and AxSYM MEIA technology analysis are shown in Figure 2.

## Discussion

The Quality control testing using all three controls in Architect CMIA and AxSYM MEIA technology using Levey – Jennings report were under range of two S.D. The CMIA have broader range of controls then MEIA technology. The new CMIA technology has higher precision and reproducibility of TnI assay and better improvement in quality of assay. Our results have shown the possibility of detection lower concentration of troponin in serum with CIMA. The similar results have got other groups (8-10). The accuracy testing, we found very good correlation between two technologies CMIA and MEIA with correlation coefficient r = 0.99. The investigation from Lam at all have found good factor of correlation too (8). The methods have correlation but great difference in mean troponin concentration patient with MI and low concentration of patients that have not myocardial infarction. We can explain it in difference of troponin mean concentration with higher analytical sensitivity ≤ 0.01 of Architect CMIA in comparison with AxSYM MEIA technology with lower analytical sensitivity ≤ 0.02. Comparison with the clinically evaluated AxSYM cTnI assay (9-11) showed that the Architect STAT TnI assay identified additional patients who have not clinical evidence of cardiac damage. This is in keeping with studies showing that the AxSYM assay may miss patients who later developed poor cardiac outcomes. Furthermore, the Architect TnI showed good agreement with the measurement at the lower end of range (9,11). At our study we have got possibility of early detection of troponin in patient serum with CMIA in the moment when the same serum was not detectable using MEIA. The measurement of CMIA 0.01-50 μg/L and MEIA 0.02-22.8 and it can explain better sensibility of CMIA technology for detection troponin in serum. The definition of high-sensitive assay would be one that had total imprecision less than 10 % at the 99th percentile. Our 99th percentile value is higher than that reported by the manufacturer (0.012 μg/L) and may be attributable to our use of freshly collected blood bank specimens.

## Conclusion

Architect CMIA Abbott technology is an applicative method in monitoring TnI in patients after myocardial infarction. In comparing methods we have got better precision and reproducibility of TnI assay for Architect STAT TnI assay technology then AxSYM MEIA technology. CMIA method is technology improved and has possibilities for detection lower and higher concentration of TnI than MEIA. The mean differences between CIMA

and MEIA methods was statistically significant for $p < 0.05$ using Student t test with very good factor of correlation $r = 0.99$. We conclude that Architect CMIA Abbott technology is method proves reliable TnI concentration in patient serum and it has better precision limits and ability to detect troponin at the low end of the range.

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